# Do frogs still get their kicks on Route 66? A transcontinental transect for amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) infection on U.S. Department of Defense installations

**Michael J. Lannoo[1] *, Christopher Petersen[2], Robert E. Lovich[3], Priya Nanjappa[4], Christopher Phillips[5], Joseph C. Mitchell[6], Irene Macallister[7]**

[1]Indiana University School of Medicine-TH, Terre Haute, IN * (mlannoo@iupui.edu); [2]Naval Facilities Engineering Command Atlantic, Norfolk, VA; [3]Naval Facilities Engineering Command-SW, San Diego, CA; [4]Association of Fish & Wildlife Agencies, Washington, DC; [5]Illinois Natural History Survey, Institute for Natural Resource Sustainability, University of Illinois, Champaign, IL; [6]Mitchell Ecological Research Service, Gainesville, FL; [7]U.S. Army Corps of Engineers, USA Construction Engineering Research Laboratory (CERL), Champaign, IL.

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One fifth of the world's amphibians now face extinction. A major factor in these declines has been the spread of infection by the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), which, as a disease (chytridiomycosis) has been devastating amphibian populations globally. To better understand the spatial and temporal scales of infection by this pathogen, we conducted a transcontinental transect for the presence of Bd. United States Department of Defense (DoD) installations were sampled from west to east along U.S. Highway 66 from California into central Illinois, and continuing eastward from there across to the Atlantic Seaboard along U.S. Interstate 64 (in sum from Camp Pendleton in California to Naval Air Station Oceana in Virginia, between $33^{\circ}$ and $39^{\circ}$ N latitude). We sampled each installation across the 2009 warm season using standardized collection and analytical techniques. This study represents the most geographically extensive survey for Bd conducted to date. Half of the amphibian species sampled (15/30) tested positive for Bd. There was a strong spatial component to our dataset; the 10 eastern temperate DoD installations had higher rates of Bd infection (18.9%) than the five bases situated in the more arid west (4.8%). There was also a strong temporal (seasonal) component to our dataset. In total, 78.5% of all positive samples were collected in the first (spring/early-summer) sampling period. These data support the conclusion that Bd is now widespread, from coast to coast, and argue that Bd, a pathogen that was once likely *epidemic*, can today be considered *endemic*, extending transcontinentally across much of North America.

## ■ Introduction

One fifth of the world's amphibians may now be facing extinction (Stuart et al. 2004; Wake and Vrendenburg 2008; http://www.iucnredlist.org/initiatives/amphibians [accessed 5 October, 2010]). In part these declines have been due to the spread of the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd; Longcore et al. 1999), which has been devastating amphibian populations on a global scale (Daszak et al. 2003; Skerratt et al. 2007; Wake and Vredenburg 2008; Kilpatrick et al. 2010). Two general scenarios, not mutually exclusive, have been proposed for the occurrence and spread of Bd; both have strong empirical support (Briggs et al. 2005; Fisher et al. 2009). The first is that Bd infection is *epidemic* (external to affected populations), spreading as a wave and wiping out individuals, populations, and species in its path. This has occurred, or is occurring, in Central America, in eastern Australia, and in parts of California (Berger et al. 1998; Lips et al. 2003, 2006; Vredenburg et al. 2010). The second scenario suggests that Bd is now *endemic* (internal to affected populations; Ouellet et al. 2005; Vredenburg et al. 2010). That is, in certain regions of the world, such as North America, much of the spread of Bd occurred decades ago (when it was epidemic). Indeed, Bd is now widespread throughout many geographic regions (Longcore et al. 2007; Fisher et al. 2009; Briggs et al. 2010) and is known to occur on every continent where amphibians reside.

Distinguishing between the epidemic and endemic hypotheses requires, in part, broad-scale studies using standardized techniques. Further, due to the confounding factor of human disturbance, it is best to examine low-impact (i.e. "natural") areas. It can be argued that the most widespread "undisturbed" habitats available in the United States today are United States Department of Defense (DoD) installations, which resist the indiscriminate human traffic experienced by parks, wildlife refuges, and other public areas. DoD installations encompass over 12 million ha and occur throughout the United States, making continental surveys possible. DoD lands are often managed differently than their surrounding landscapes, using ecosystem management techniques. Indeed, American military lands harbor some of the greatest concentrations of endangered and threatened habitats and species in the United States (Stein et al. 2008).

We conducted a transcontinental transect designed to assess the presence of Bd. DoD installations were sampled from west to east along U.S. Highway 66 (the "Mother Road") from California into central Illinois, and continuing eastward from there across to the Atlantic Seaboard along U.S. Interstate 64 (in sum from Camp Pendleton in California to Naval Air Station Oceana in Virginia, between 33° and 39 N latitude). We sampled across warm seasons, and used standardized collection and analytical techniques to address the following questions: 1) Does Bd occur in amphibian populations in these relatively undisturbed environments? 2) Is there a spatial pattern to the presence of Bd? 3) Is there a temporal pattern to the presence of Bd? and 4) Do our results shed light on whether Bd is acting as an epidemic or endemic infection across North America?

## ■ Materials and Methods

In 2009, a total of 15 DoD installations were sampled as follows (Fig. 1; from west to east): Marine Corps Base Camp Pendleton in California, Camp Navajo in Arizona, Kirtland and Cannon Air Force Bases in New Mexico, Fort Sill and Camp Gruber in Oklahoma, Fort Leonard Wood in Missouri, Sparta Training Center in Illinois, Naval Support Activity Crane in Indiana, Fort Knox in Kentucky, and Radford Army Ammunitions Plant, Fort Lee, Fort A.P. Hill, Fort Belvoir, and Naval Air Station Oceana in Virginia). Each base was sampled three times: once in the spring/early summer (April, May, or the first week in June), once in mid-summer (July, August), and once in the late summer/fall (September, October). Generally, three wetland sites were sampled at each installation. All animals were handled using sterile techniques and sampled using cotton-tipped swabs following protocols outlined in Skerratt et al. (2008) and Pessier and Mendelson (2010). Swabs were analyzed for Bd using conventional PCR (polymerase chain reaction; Annis et al. 2004; Pessier and Mendelson, 2010). Positive and negative controls were run with each sample; samples were analyzed twice.

Mean annual temperature and precipitation data for a 30-yr period (1971–2000) were obtained from stations near or at each base by searching National Oceanic and Atmospheric

Administration (NOAA) databases (http://cdo.ncdc.noaa.gov/cgi-bin/climatenormals/climatenormals.pl)..

From among our three datasets (Bd infection rate [arcsine transformed], temperature, and precipitation) only temperature data were normally distributed (Shapiro-Wilk normality test, Program R) and therefore we used nonparametric Chi-square ($\chi^2$) and Kruskal Wallis tests (SPSS v. 17) for our analyses. A $\chi^2$ goodness of fit test was used to compare observed Bd infection rates across seasons (Spring/Early Summer, Mid-Summer, and Late Summer/Fall) to expected rates (based on total rate of Bd infection). Kruskal Wallis tests were used to compare Bd infection rates, temperatures, and precipitation values between arid and temperate installations. Arid installations were defined as the five western-most bases (Camp Pendleton, Camp Navajo, Kirtland Air Force Base AFB, Cannon AFB, and Ft. Sill); temperate bases were Camp Gruber, Ft. Leonard Wood, Sparta Training Center, NSA Crane, Ft. Knox, Radford AAP, Ft. A.P. Hill, Ft. Belvoir, Ft. Lee, and NAS Oceana. Significance levels were set at $p \leq 0.05$

## ■ Results

In total, from all bases during all visits, 1,306 amphibians were sampled; 217 (16.6%) swabs tested positive for Bd. We did not detect Bd at two bases, Camp Navajo, Arizona and Fort Sill, Oklahoma. This was not due to sample size, per se. Thirty five samples were taken at Camp Navajo; 34 in July (mid-summer), 1 in September (late-summer/fall). At Fort Sill, a total of 43 samples were taken; 12 during June (mid-summer), 31 during September (late-summer/fall). This result could have been due, in part, to a lack of samples during the spring/early summer sampling period (due to cold and snow), when the majority of positive samples at other bases were collected (see below).

Bd was detected at the remaining 13 bases (Fig. 2). Infection rates among these sites ranged from 2% (1 of 46 samples positive) at Kirtland Air Force Base in New Mexico to 39% (7 of 18 samples positive) at Fort Belvoir in Virginia. Other sites with high percentages of positive samples included Sparta Training Center in Illinois (31%; 55 of 180 samples

positive), Camp Pendleton in California (26%; 5 of 19 samples positive), and Radford Army Arsenal in Virginia (25%; 15 of 60 samples positive). Sparta had the highest absolute number of positive samples (55), Fort Leonard Wood had the second highest (38).

### *Species*

Species infected with Bd covered a wide phylogenetic range including: four species of plethodontid salamanders (*Desmognathus fuscus*, *Eurycea cirrigera*, *Eurycea longicauda*, and *Pseudotriton ruber*), three species of toads (*Anaxyrus americanus, Anaxyrus fowleri, Anaxyrus woodhousii*), five hylid species (*Acris blanchardi, Acris crepitans*, *Hyla cadaverina*, *Hyla chrysoscelis*, and *Pseudacris crucifer*), and four ranid species (*Lithobates catesbeianus, Lithobates clamitans, Lithobates palustris,* and *Lithobates sphenocephalus*). At no point during this study did we observe dead or dying amphibians.

### *Spatial Pattern*

Aridity affected Bd infection rates. Five of the six sites with the lowest infection rates (Camp Navajo, 0%; Fort Sill, 0%; Kirtland Air Force Base, 2%; Cannon Air Force Base, 6%, and Camp Gruber, 8%) occur in the arid southwest (Arizona, New Mexico) or on the Great Plains (Oklahoma); the exception was Fort Lee (7%) in Virginia. Remaining sites occur in coastal areas, or inland areas that receive higher levels of precipitation. A second way we explored this trend was to compare the data for the western arid bases (Pendleton, Navajo, Kirtland, Cannon, and Ft. Sill) to the data for the eastern temperate sites. The rate of positive samples for the arid installations was 4.8% (10/208); the rate for the eastern temperate sites was 18.9% (207/1098), a statistically significant difference (Kruskal-Wallis, $p = 0.027$). Rates of precipitation were also different between the western arid and eastern temperate bases ($x = 47.9$ vs. 110.6 cm annually; Kruskal-Wallis, $p = 0.002$), although temperatures were not ($x = 14.3$ vs. 13.6° C; Kruskal-Wallis, $p = 0.3$).

### *Temporal Pattern (Seasonality)*

There was a strong seasonal component to our results (Fig. 3), which was statistically significant ($\chi^2$, $p = 0.031$). During the spring/early-summer sampling period, 39.3% of all samples were positive. This number dropped to 6.1% for mid-summer samples, and to 4.5%

for late-summer/fall samples. Most bases followed this pattern, although three East-Coast installations—Fort A.P. Hill, Fort Lee, and Naval Air Base Oceana—had higher percentages of animals infected during the late-summer/fall than in the mid-summer period.

## ■ Discussion

Bd was detected in 13 of 15 DoD installations, transcontinentally. Fifteen of 30 amphibian species sampled tested positive for Bd. There were both spatial and temporal patterns to Bd infection rates, as follows.

### *Bd is found in the highly secure environments of U.S. DoD installations*

In aggregate, the data for all bases over all three sampling periods (spring/early-summer, mid-summer, late-summer/fall) show a 16.6% rate of Bd infection. Bd was found in all but two installations, Camp Navajo in Arizona and Fort Sill in western Oklahoma. Lack of Bd detection on these bases may be the result of insufficient sampling during the first sampling period due to inclement weather (cold). Amphibians were not active during the first sampling period at either of these bases and spring and early-summer was the time when Bd was most likely to be detected (79% of our positive samples came from this first sampling period; see below).

We sampled about 10% (30/298) of all known United States species and found Bd in half of them. While Bd absence in the remaining species may be due to inherent resistance (Woodhams et al 2007b) or ecological avoidance (Lips et al. 2003), it is most probable that in cases of no detection, individuals sampled happened to be negative, or to test negative at the time of sampling. It is likely that all amphibian species are susceptible to Bd infection, although species-specific variation in susceptibility has been shown (Woodhams et al. 2007a), as has intraspecific variation in susceptibility (Tennessen et al. 2009). Several of the species that tested positive have been documented as Bd-positive in other studies; salamanders and ranids, including Bullfrogs, may be carriers of this infection (Daszak et al. 2004).

### *There is a spatial pattern to the presence of Bd*

The ten eastern temperate DoD installations had significantly ($p = 0.027$) higher rates of Bd infection (19.6%; 207/1098) than the five bases situated in the arid western ecosystems (4%; 10/208). Bd went undetected at two of these bases (Camp Navajo and Fort Sill); two arid bases each had single-digit levels of detection (Kirtland, 2%, Cannon, 6%). Camp Pendleton was the exception. It had a 26% rate of Bd infection, but this installation is coastal and subject to ground fog. Bd is known to favor cool, moist conditions (Ribas et al. 2009; Fisher et al. 2009). It therefore follows that warm and dry (i.e., arid) conditions inhibit this pathogen. Our data are consistent with this interpretation (Fig. 2).

### ***There is a temporal (seasonal) pattern to the presence of Bd***

There was a strong temporal component to our dataset (Fig. 3). In total, 78.5% of all positive samples came in the first (spring/early-summer) sampling period, and broken out by sampling period, the percent positive samples were 39.3% (168 of 427), 6.1% (29 of 477), and 4.5% (17 of 374). The data for the majority of bases (Camp Pendleton, Cannon Air Force Base, Camp Gruber, Fort Leonard Wood, Sparta Training Center, Crane Naval Surface Warfare Center, Fort Knox, and Fort Lee) followed this temporal pattern. Overall, our data suggest a strong seasonal component to Bd infection, with the earliest sampling period showing the greatest infection rate (Fig. 3).

Seasonality in Bd infection rates has been previously demonstrated (Berger et al. 2004; Gaertner et al. 2009). As summer proceeds, Bd-positive frogs appear to loose their infection (Woodhams et al. 2003; Piotrowski et al. 2004; Pessier and Mendelson 2010; Richards-Zawacki 2010). Infected animals can also develop chytridiomycosis and die, and thus be lost to later surveys. Just as we suggest the spatial pattern of Bd presence is due to variation in moisture levels (with moisture promoting infection rates), we suggest the temporal (seasonal) pattern is due to moisture availability, with Bd present at the highest rates during the wettest times of the year. Inverting our view, our data suggest that Bd rates are lower in arid areas (arid deserts and the Great Plains) and during drier times of the year (mid- to late-summer and fall). Temperature may be a covariate, with cooler temperatures promoting the infection, although in our study, by controlling for latitude we controlled, as must as possible in a continental transect, for temperature.

### *Bd is an endemic infection across much of the United States middle latitudes*

These data support the conclusion of Ouellet et al. (2005) that Bd is now widespread across much of North America. This spatial pattern—from coast-to-coast—argues for an infection that, while once was likely *epidemic*, today is *endemic*. Further, the phylogenetic range of species that presented positive suggests that this infection has been associated with ecosystems long enough to infect numerous species. There are known to be pockets of wilderness, for example in regions of the Sierra Nevada, where Bd has yet to reach (Vredenberg et al. 2010). At these places, when Bd arrives it is predicted to be an *epidemic* infection, and we suspect amphibian extirpations will follow.

## ■ Conclusions

This study represents the most geographically extensive survey for Bd conducted to date. Half of the amphibian species surveyed (15/30) tested positive for Bd as follows: Plethodontidae (four species), Bufonidae (three species), Hylidae (five species) and Ranidae (three species). There was a strong spatial component to our dataset. The ten eastern temperate DoD installations had higher rates of Bd infection (18.9%) than the five bases situated in the arid southwest or Great Plains ecosystems (4.8%). There was also a strong temporal (seasonal) component to our dataset. In total, 78.5% of all positive samples came in the first (spring/early-summer) sampling period. These data support the conclusion of Ouellet et al. (2005) that Bd is now widespread across much of North America and suggests that Bd, an infection that, while once was likely *epidemic*, today can be considered *endemic*, occurring transcontinentally, even on the relatively undisturbed lands managed by the DoD. Do frogs still get their kicks on Route 66? Yes, and amphibians will probably persist as long as the relatively high seasonal Bd infection rates temperate populations experience remain largely subclinical.

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## ■ References

Annis SL, Dastoor FP, Ziel H, Daszak P, Longcore JE (2004) A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. J Wildl Dis 40: 420–428.

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H (1998) Chytridiomycosis causes amphibian mortality associated with population decline in the Rain Forests of Australia and Central America. PNAS 95: 9031–9036.

Berger L, Speare R, Hines HB, Marantelli G, Hyatt AD, McDonald KR, Skerratt LF, Olsen V, Clarke JM, Gillespie G, Mahony M, Sheppard N, Williams C, Tyler MJ (2004) Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Aust Vet J 82: 434–439.

Briggs CJ, Vredenburg VT, Knapp RA, Rachowicz LJ (2005) Investigating the population-level effects of chytridiomycosis: An emerging infectious disease of amphibians. Ecology 86: 3149–3159.

Briggs CJ, Knapp RA, Vredenburg VT (2010) Enzootic and epizootic dynamics of the chytrid fungus pathogen of amphibians. PNAS. pnas.0912886107.

Daszak P, Cunningham AA, Hyatt AD (2003) Infectious disease and amphibian population declines. Divers Dist 9: 141–150.

Daszak P, Strieby A, Cunningham AA, Longcore JE, Brown CC, Porter D (2004) Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herp J 14: 201–207.

Fisher, MC, Garner TWJ, Walker SF (2009) Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomcosis in space, time, and host. Annu Rev Microbiol 63: 291–310.

Gaertner JP, Gaston MA, Spontak D, Forstner MR, Hahn D (2009) Seasonal variation in the detection of *Batrachochytrium dendrobatidis* in a Texas population of Blanchard's Cricket Frog (*Acris crepitans blanchardi*). Herp Rev 40: 184–187.

Kilpatrick AM, Briggs CJ, Daszak P (2010) The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends Ecol Evol 25: 109–118.

Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Carey C, Livo L, Pessier AP, Collins JP (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Ecology 103: 3165–3170.

Lips KR, Reeve JD, Witters LR (2003) Ecological traits predicting amphibian declines in Central America. Cons Biol 17: 1078–1088.

Longcore JR, Longcore JE, Pessier AP, Halteman WA (2007) Chytridiomycosis widespread in anurans of northeastern United States. J Wildl Manage 71: 435–444.

Longcore J, Pessier A, Nichols DK (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91: 219–227.

Ouellet M, Mikaelian I, Pauli BD, Rodrigues J, Green DM (2005) Historical evidence of widespread chytrid infection in North American amphibian populations. Cons Biol 19: 1431–1440.

Pessier, AP, Mendelson III, JR (2010) A manual for control of infectious diseases in amphibian survival assurance colonies and reintroduction programs. Proceedings from a Workshop 16–18 Feburary 2009, San Diego Zoo, San Diego, California, USA.

Piotrowski JS, Annis SL, Longcore JE (2004) Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96: 9–15.

Ribas L, Li M-S, Doddington BJ, Robert J, Seidel JA, Kroll JS, Zimmerman LB, Grassly NC, Garner TWJ, Fisher MC (2009) Expression profiling the temperature-dependent amphibian response to infection by *Batrachochytrium dendrobatidis*. PLoS One 4: e8408.

Richards-Zawacki CL (2010) Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian Golden Frogs. Proc Royal Soc Biol Sci B 277: 519–528.

Skerratt LF, Berger L, Hines HB, McDonald KR, Mendez D, Speare R (2008) Survey protocol for detecting chytridiomycosis in all Australian frog populations. Dis Aquat Org 80: 85–94.

Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N (2007) Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4: 125–134.

Stein BA, Scott C, Benton N (2008) Federal Lands and Endangered Species: The Role of Military and Other Federal Lands in Sustaining Biodiversity. BioScience 58: 339-347.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller, RW (2004) Status and trends of amphibians and extinctions worldwide. Science 306: 1783–1786.

Tennessen JA, Woodhams DC, Chaurand P, Reinert LK, Billheimer D, Shyr Y, Caprioli RM, Blouin MS, Rollins-Smith LA (2009) Variations in the expressed antimicrobial peptide repertoire of Northern Leopard Frog (*Rana pipiens*) populations suggest intraspecies differences in resistance to pathogens. Develop Comp Immunol 33: 1247–1257.

Vredenburg VT, Knapp RA, Tunstall TS, Briggs CJ (2010) Dynamics of an emerging disease drive large-scale amphibian population extinctions. PNAS. pnas.0914111107.

Wake DB, Vredenburg VT (2008) Colloquium paper: are we in midst of the sixth mass extinction? A view from the world of amphibians. PNAS 105: 11466–11473.

Woodhams DC, Hyatt AD, Boyle DG, Rollins-Smith LA (2007a) The Northern Leopard Frog *Rana pipiens* is a widespread reservoir species harboring *Batrachochytrium dendrobatidis* in North America. Herpetol Rev 39: 66–68.

Woodhams DC, Alford RA, Marantelli G (2003) Emerging disease of amphibians cured by elevated body temperature. Dis Aquat Org 55: 65–67.

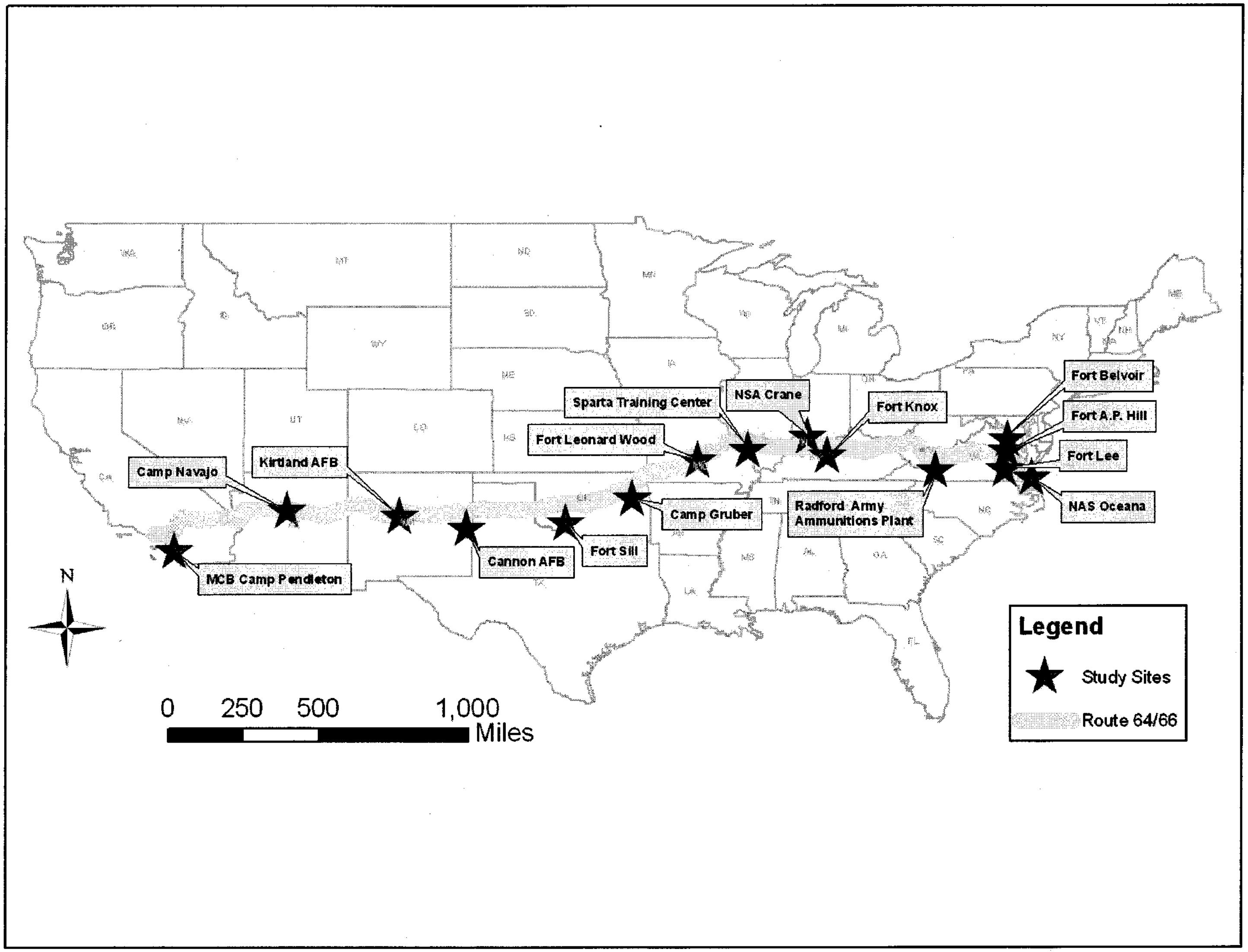
Woodhams DC, Ardipradja K, Alford RA, Harris R, Marantelli G, Reinert LK, Rollins-Smith LA (2007b) Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. Anim Cons 10: 409–417.

## Figure Legends

Figure 1. Department of Defense installations participating in the present study. From California to Illinois, bases were located near Route 66; from Illinois east to the coast, sites were chosen near Interstate 64 to hold latitude relatively constant (between 33° and 39° N).

Figure 2. Percentage of Bd positive samples by installation. Bases are arranged from west to east, in the order they appear in Figure 1. Right side y-axis indicates both mean annual temperature (° C, yellow line) and mean annual precipitation (cm, red line). Note the low percentage of positive samples from the arid western installations, although the relationship between annual precipitation levels and Bd infection rates was not statistically significant.

Figure 3. Temporal pattern (seasonality) of Bd infection rates across all installations. Note the strong tendency for the highest infection rates to occur during the spring/early summer sampling period, followed by a precipitous drop off during the mid- to late-summer and fall.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[1]Indiana University School of Medicine-TH, Terre Haute, IN * (mlannoo@iupui.edu); [2]Naval Facilities Engineering Command Atlantic, Norfolk, VA; [3]Naval Facilities Engineering Command-SW, San Diego, CA; [4]Association of Fish & Wildlife Agencies, Washington, DC; [5]Illinois Natural History Survey, Institute for Natural Resource Sustainability, University of Illinois, Champaign, IL; [6]Mitchell Ecological Research Service, Gainesville, FL; [7]U.S. Army Corps of Engineers, USA Construction Engineering Research Laboratory (CERL), Champaign, IL.

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